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Alignment of direct detection device micrographs using a robust Optical Flow approach

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ABSTRACT

The introduction of direct detection devices in cryo-EM has shown that specimens present beam-induced motion (BIM). Consequently, in this work, we develop a BIM correction method at the image level, resulting in an integrated image in which the in-plane BIM blurring is compensated prior to particle picking. The methodology is based on a robust Optical Flow (OF) approach that can efficiently correct for local movements in a rapid manner. The OF works particularly well if the BIM pattern presents a substantial degree of local movements, which occurs in our data sets for Falcon II data. However, for those cases in which the BIM pattern corresponds to global movements, we have found it advantageous to first run a global motion correction approach and to subsequently apply OF. Additionally, spatial analysis of the Optical Flow allows for quantitative analysis of the BIM pattern. The software that incorporates the new approach is available in XMIPP (http://xmipp.cnb.csic.es).

1. Introduction

The single-particle analysis (SPA) technique is able to yield three-dimensional (3D) structural information for biological complexes at near atomic resolution by combining many thousands of projection images obtained using transmission electron microscopy (TEM) (Frank, 1996). To achieve high-resolution results in SPA, the characteristics of the image-recording medium are of great importance. Traditionally, electron microscopy images were either recorded on photographic film or with scintillator-based charge-coupled device (CCD) cameras. Each of these two types of detectors offered certain advantages and disadvantages. Film was the preferred recording medium for high-resolution information but required manual scanning of the micrographs, thus limiting automation of processing. Scintillator-coupled CCDs permitted high-throughput image acquisition, allowing full integration between the electron microscope and image-processing software packages. However, scintillator-coupled CCDs record photons, not electrons, and the conversion of electrons to photons comes at the expense of resolution loss at high spatial frequencies (Frank, 2006). This "status quo" limitation has been overcome recently by the new generation of "direct detection devices" (DDDs), first introduced as academic prototypes in 2005 (Milazzo et al., 2005) and offered commercially in 2010 (Jin and Bilhorn, 2010). These sensors detect electrons directly and provide sharper images and higher signal-to-noise ratios (SNRs) (Bammes et al., 2012). Additionally, the fast image acquisition rate of these DDD detectors, ranging from 16 to 400 images per second (Bai et al., 2013; Bammes et al., 2012; Li et al., 2013b), makes it possible to study the behavior of frozen hydrated specimens as a function of electron dose and rate. Therefore, it has become clear that biological specimens in a solid matrix of amorphous ice move during imaging, resulting in "beam- induced motion" (BIM) (Brilot et al., 2012), which is a critical experimental "resolution barrier" in cryo-electron microscopy (Glaeser and Hall, 2011). The subsequent introduction of DDDs has cleared a path to obtaining reconstructions at close-to-atomic resolution for a broad range of specimens. However, the number of reported works that use DDDs is currently not large, and therefore, certain basic questions on BIM characterization remain unanswered. In general, BIM is expected to induce patterns of local movement, although the degree of locality and the

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extent of the movement itself are varying widely in different reports (Bai et al., 2013; Booth et al., 2004; Campbell et al., 2012; Li et al., 2013a,b).

In Bai et al. (2013), results for the Falcon II (FEI) are presented using two test samples: the prokaryotic and eukaryotic ribosome. The authors report a movie processing workflow in which an initial 3D map (which disregards BIM) is first obtained and subsequently used to estimate an initial alignment from different frames by applying a statistical refinement method (Scheres, 2012). In their work, the authors report a high degree of local sample movements (see Fig. 1b in Bai et al. (2013)). The algorithm proposed in Bai et al. (2013) produces high-resolution information, but it requires the specimen images to be detected and picked up from the initial video frames, which is a challenge for small particles. However, in Li et al. (2013b), the authors showed results for the K2 Summit (Gatan) direct electron-detection camera and achieved ~3.6-Å resolution in their 3D map of an archaeal 20S proteasome (~700 kDa and dihedral D7 point group symmetry) using 10,000 particles. The alignment method used in Li et al. (2013b) consists of a pure in-plane drift correction in which a step of the sub-frame translational alignment is introduced by dividing each frame into a number of sub-frames (normally 3×3 sub-frames, each of 2000×2000 pixels). This approach is fast if running on GPUs (typically, it takes approximately 10–20 s to process 16 frames of 3876×3876 pixels), and at the end, an "average" micrograph is generated for each movie via the summation of all corrected frames. The output is easy to connect with standard processing workflows in use in the field because the DDD "video" is transformed into a "micrograph". The method is certainly appropriate for global sample movements but is not the best option if the sample motion is local. However, by achieving close to atomic resolution, Li et al. (2013b) convincingly showed that for their data, the majority of the motion to be corrected was global, especially if the first few frames were discarded (see Fig. 4b and e in Li et al. (2013b)). At the same time, we note the difference from the results in Bai et al. (2013) and Campbell et al. (2012) in which the authors report a high degree of local sample movements, as previously mentioned. Recently, in Scheres (2014), another method for BIM compensation was introduced that operates over sets of previously picked particles by fitting them to line trajectories along the frames of the stack. Moreover, Wang et al. (2014) introduced an approach that corrects the motion of boxed particles using the running averages of the frames and calculating the cross-correlations between each frame and the sum of the previously aligned frames, starting at the end of the exposure and working backwards towards the beginning. In this work, we do not enter into a discussion of the nature of BIM itself, but instead, we concentrate on a new image- processing approach that aims to achieve the following objectives: (1) obtain an in-plane "BIM corrected" image that integrates all frames and is computed directly from the stack without performing a particle picking step, and (2) provide fast, objective and quantitative characterization of BIM that accounts for both global and local BIM patterns. Naturally, this integrated image can be used in any of the standard image-processing workflows in cryo-EM as if it were a traditional micrograph.

The proposed method is based on an advanced Optical Flow approach (abbreviated as "OF" in this work) using a pyramidal implementation of the Lucas–Kanade (LK) algorithm (Lucas and Kanade, 1981) with iterative refinement (Bouguet, 2001), which makes the approach quite robust to high levels of noise (Vargas et al., 2014). In essence, OF works best at a local level and is therefore particularly suited for those cases in which the BIM pattern presents a high degree of local movements, as in the Falcon II data sets used in this work. If the BIM pattern is characterized primarily by global movements, OF will have only a minor effect on the final average. Still, even for those latter cases, we have found it advantageous to use the Li et al. (2013b) method combined with OF, by running the Li method followed by the second method to obtain an additional level of refinement and a highly intuitive graphical representation of the total BIM pattern.

2. Methods

Our proposed method is based on a regularized Optical Flow approach. The input is a video composed of a set of unaligned low dose frames, and the output is a single image obtained by averaging the resulting motion-corrected frames.

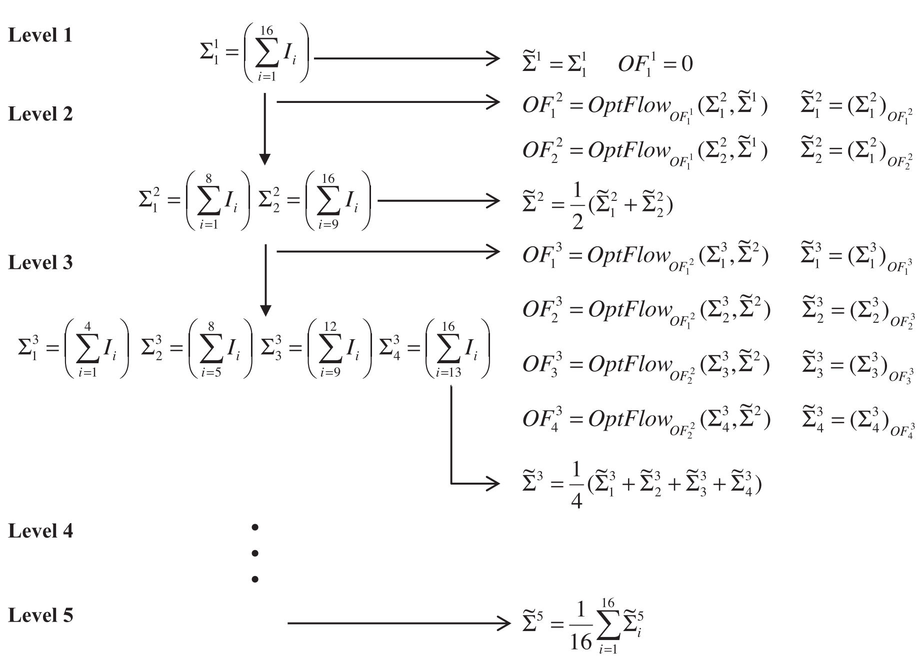

Fig.1. Diagram of the proposed Optical Flow method: The required steps for alignment of a video with 16 frames using the proposed Optical Flow method.